

A study assessing some metal elements contamination levels in grasscutter (*Thryonomys swinderianus* Temminck) meat

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Abstract. Chromium, Arsenic and Nickel contamination levels in fresh and smoke-dried grasscutters were assessed in this study. Muscle, liver and kidney of wild and domesticated grasscutters were purchased from Uwa, New Benin, Arbico markets and Makarios graduate grasscutter farmers Edo Development and Property Authority Housing Estate respectively, in Benin City, Edo State, Nigeria and analysis was carried out using Atomic absorption spectrophotometer (AAS) after wet digestion of samples with 1:3 Perchloric acid and Nitric acid. Data obtained were statistically analyzed using SAS. In a 4x3x2 randomized complete block design. The ranges obtained for the heavy metals analyzed in (fresh and smoke-dried) muscle, liver and kidney samples were observed as follows: Cr (0.072 to 1.186 mg/kg) (0.074 to 0.306 mg/kg), Cd (0.186 to 7.516 mg/kg) (0.277 to 2.723 mg/kg) and Ni (0.041 to 0.725 mg/kg) (0.045 to 0.188 mg/kg) respectively. Generally, the heavy metal concentrations were significantly higher ($P < 0.05$) in the fresh and smoke-dried muscle, liver and kidney of wild grasscutters than the values from domesticated grasscutters. Fresh grasscutter samples were however, significantly higher ($P < 0.05$) in these heavy metals than in smoke-dried grasscutter samples. Chromium and Nickel levels were higher than recommended accepted limits. Arsenic (As) was not detected in all samples analyzed.

Keywords: Arsenic, contamination, chromium, domestication, grasscutter, nickel.

INTRODUCTION

Bush meat is a general term applied to meat obtained from any wild terrestrial mammal, bird, reptile or amphibian harvested for subsistence or trade, mostly illegally. Since the beginning of civilization, game meat has been a main source of meat for humans and the main activity was hunting, particularly in developing countries (Cawthorn and Hoffman, 2015; Ebabhamiegbebho and Ohanaka, 2012; Asibey, 1971; Baptist and Mensah, 1986). It is popular with a lower concentration of lipid than farm animals. Several studies have demonstrated that various types of game meat (deer, reindeer, antelopes, buffaloes among others) contain low levels of fat and a higher proportion of polyunsaturated (omega-3) fatty acids compared with

meat from conventional species like beef, lamb and pigs. To lower the intake of saturated fat and increase the intake of polyunsaturated fat is frequently recommended from a health perspective mainly to prevent cardiovascular diseases and cancer. The properties of game meat as a result of animals grazing natural pasture vegetation rich in omega-3 fat and antioxidants such as vitamins C and E. Carotenoids and flavonoids create nutritional advantages in this aspect (Wiklund and Malmfors, 2012). Due to the continuous environmental pollution in chemical residues including heavy metals are now more and more present in game meat. Toxic metals, which enter organisms by binding to the enzymatic systems from animal cells or to some cell membrane

components, cause specific intoxication and in certain situations show their carcinogenic, teratogenic or embryotoxic potential (Mitranescu et al., 2011). The bush meat variety that is of significance in this study is grasscutter (*Thryonomys swinderianus*). The meat obtained from grasscutter is regarded as a delicacy in a number of West African countries (Ebabhamiegbeho and Ohanaka, 2012). For this reason, grasscutter meat is more expensive than mutton, chicken, fish and beef. There is a general wide acceptability of grasscutter regardless of religious faith and social status (Yaro et al., 2012).

Meat and meat products form an important part of the human diet as well as an important source of a wide range of nutrients, but they may also contain certain toxic substances. Although the level of these toxic substances in muscle is generally low, offal, such as liver and kidney, showed higher concentration of toxic substances than most other foods (Khalafalla et al., 2011). As a wild herbivorous rodent, the grasscutter is widely distributed and is a valuable animal in West Africa where grass provides its main habitat and food. Within the West African sub-region, grasscutter is the favorite bush meat species and accounts for the greater proportion of bush meat sold in markets (Falconer, 1992; Ntiama-Baidu, 1998). Its distribution is therefore, determined by the availability of adequate or preferred grass species for food (NCR, 1991). Intensive production of grasscutters has been undertaken in countries such as Benin Republic and Togo and agricultural extension services in Cameroon, Cote' D'voire, Gabon, Ghana, Nigeria, Senegal and Zaire have also encouraged farmers to rear these rodents in rural and peri-urban areas (Asibey, 1971; Baptist and Mensah, 1986).

One of the most important aspects of environmental pollution to humans is the intake of toxic substances present in food (Orisakwe et al., 2012; JECFA, 2002). Meat consumption is increasingly becoming a larger part of diet worldwide. However, the bioaccumulation of toxic metals from anthropogenic pollution is increasingly becoming a potential health risk to humans (Ihiedioha et al., 2014). Those of particular importance in this study are Arsenic (As), Chromium (Cr) and Nickel (Ni). This is consequent upon the fact that metal pollution, from historical and ongoing industrial mining and agricultural activities, is one of the greatest environmental threats in the world (Roggeman et al., 2014). The metalloid, Arsenic (As) has been classified as the most hazardous substance in the environment (ATSDR, 2011), thus raising concerns as to how hygienic the grasscutter meat processing environment and personnel are. The toxicity of As is largely related to the intake of As III and As V which, in acute doses, can lead to multiple organ failure. A chronic, long-term ingestion of these with As-contaminated water has been associated with a range of health effects from skin lesions, cancer in the lung, bladder, kidney and skin, cardiovascular diseases, neuro-

toxicity, developmental toxicity, abnormal glucose metabolism and diabetes (Molin et al., 2015; EFSA, 2009). A recent study which monitored consumption of cow meat in an urban Nigerian population reported that Chromium level intakes were above the recommended daily intake (RDI) set by the WHO/FAO. Closely related to Chromium is Nickel, which is also carcinogenic to humans and have been shown to be potential inducers of kidney and lung tumors in experimental animals (De Medeiros et al., 2008; Lee, 2006; Lee, 2001) and although varying levels have been reported recently (Mahmoud and Abed-Moshein, 2015; Oforka et al., 2012), it is also necessary to also evaluate its level in grasscutter meat and compare to internationally-approved limits as available literature in this regard is scarce.

Several reports indicate that game meat can be an important source of trace metals because of its increasing availability mainly due to increased hunting activity (Ramanzin et al., 2010). Single metals are higher in animals living near mining sites or other sources of pollution (Mankovska and Steinnes, 1995; Reglero et al., 2009; Pokomy et al., 2009). Since grasscutter, a major source of bush meat is consumed in Africa and other parts of the world, it becomes imperative to ascertain the levels of some of these metals and make appropriate recommendations. This information will also guide prospective marketers, processors and consumers as to where they buy this game meat from, what to observe and possibly how best to process them. The broad objective of this study therefore is to evaluate the levels of Cr, As and Ni in the liver, muscle and kidney of fresh and smoke-dried grasscutter samples obtained from wild and domesticated sources.

METHODOLOGY

Sample collection

Fresh and smoke-dried wild grasscutter meats were purchased from game meat processing centres in Uwa, New Benin and Arbico markets in Benin City, Edo State, Nigeria. Fresh domesticated grasscutter meats were also purchased from Makarios graduate grasscutter farmers society in Edo Development and Property Authority Housing Estate, Ugbowo, Benin City, Edo State and smoke-dried. The sex, age and feeding habits of grasscutter were not taken into consideration during the sampling. A total of 24 random grasscutter samples were collected in a 6-month period. Randomized complete block design (Two Way ANOVA), was used in a 4x3x2 factorial arrangement to assess whether heavy metals varied significantly between liver, kidney and muscle of the animal. The samples were collected in separate polyethylene bags and transported to the laboratory for analysis.

Table 1. Mean concentrations of chromium (mg/kg) in fresh kidney, liver and muscle of grasscutters from different locations on dry matter basis.

Variable	Location	Organs			SEM
		Kidney	Liver	Muscle	
Cr	DC	0.137 ± 0.004 ^a _D	0.138 ± 0.002 ^a _D	0.072 ± 0.003 ^b _D	0.00000366
	NB	0.190 ± 0.004 ^c _C	0.589 ± 0.009 ^b _B	1.186 ± 0.006 ^a _A	0.0000189
	U	0.309 ± 0.008 ^a _B	0.270 ± 0.010 ^b _C	0.148 ± 0.003 ^c _C	0.000027
	AB	0.427 ± 0.004 ^b _A	0.760 ± 0.010 ^a _A	0.246 ± 0.004 ^c _B	0.0000203
	SEM	0.0000127	0.0000325	0.00000707	

Location: DC, Domesticated; NB, New Benin Market; U, Uwa Market; AB, Arico Market. SEM: Standard Error of Mean. a, b, c, superscripts within rows indicated organs are statistically significant. A, B, C, D subscripts within columns indicated locations are statistically significant. Mean with different scripts are significantly different at $P < 0.05$. Mean with the same scripts are not significantly different at $P > 0.05$.

Sample preparation

The collected samples were decomposed by wet chemical digestion method for determination of various minerals. In the laboratory, 1 g of the samples (liver, kidney and muscle) was weighed into the digestion flask. To each portion of sample in the flask, 5 ml of perchloric acid and 15 ml of 0.1 N concentrated HNO_3 in a ratio 1:3 were added and then heated in an electric plate until sample became clear. After digestion, 5 ml of 20% HCl (0.1 N) was added to the content. The content of the flask was filtered using Whatman filter NO42 paper into a 100 ml volumetric flask and was made up to the mark with a distilled water and then stored in a plastic reagent bottle, ready for Atomic Absorption Spectroscopy (AAS) analysis. In the laboratory, 5 g of the fresh and smoke-dried samples (liver, kidney and muscle) was also weighed in separate beakers and oven dried at 100°C to constant weight for determination of moisture content.

Sample analysis

Chromium (Cr), Arsenic (As) and Nickel (Ni) levels in fresh and smoke-dried liver, kidney and muscle samples of standard solutions were analyzed Using AAS MODEL-SOLAAR 969 UNICAM Series (Atomic Absorption Spectrophotometer). Metal ion in a solution is converted to atomic state by means of a flame. Light of the appropriate wavelength is supplied and the amount of light absorbed can be measured against a standard curve (Haswell, 1991). The source of radiation was a Hollow lamp, which contained a cathode constructed of same metals as were analyzed. This emits the wavelength characteristic of the metal, and a different lamp was used for each metal. The light from the lamp was directed through a flame (Acetylene-Air flame) unto a monochromatic, which selected the preferred wavelength. The light monochromatic was detected by a photomultiplier tube and converted to an electrical signal. The sample was aspirated in the flame where the solution was evaporated and the metal-containing compounds

were volatilized and dissociated into ground state atoms. ground state atoms absorbed the radiation from the hollow lamp and excited to higher energy levels. The absorbance value for each metal in the sample was recorded directly from the spectrophotometer and the concentration of each was determined. Determinations of the tested elements in all samples were carried out using AAS MODEL-SOLAAR 969 UNICAM SERIES spectrophotometer at wavelength (nm), for Chromium (357.8), Arsenic (193.7) and Nickel (232.0).

Statistical analysis

Data obtained from this study were statistically analyzed using Genstat software (2009) to establish the results. Data are shown as the mean \pm standard error. Metal concentrations in fresh and smoke-dried samples were analyzed with respect to locations/origin. A statistical level of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Chromium

Chromium concentrations (mg/kg) was significantly higher ($P < 0.05$) in the muscle of fresh grasscutters sold at New Benin market than in other locations (Table 1). On the other hand in the smoke-dried grasscutters, Cr concentrations (mg/kg or ppm) was significantly higher ($P < 0.05$) in the muscle of those sold at New Benin market than the liver and kidney (Table 2). Cr concentration was higher in the fresh than in the smoke-dried samples. The highest concentration for chromium was observed in the fresh and smoke-dried muscle of grasscutters (1.186 and 0.306 ppm) from New Benin market location respectively, while the lowest concentration of (0.072 and 0.074 mg/kg) from the domesticated location respectively, was found in the fresh and smoke-dried muscle of grasscutters.

Chromium concentrations as observed in the fresh

Table 2. Mean concentrations of chromium (mg/kg) in dried kidney, liver and muscle of grasscutters from different locations on dry matter basis.

Variable	Location	Organs			SEM
		Kidney	Liver	Muscle	
Cr	DC	0.121 ± 0.003 ^{b_D}	0.157 ± 0.002 ^{a_B}	0.074 ± 0.004 ^{c_D}	0.0000042
	NB	0.167 ± 0.002 ^{c_B}	0.255 ± 0.004 ^{b_A}	0.306 ± 0.001 ^{a_A}	0.0000356
	U	0.182 ± 0.003 ^{a_A}	0.186 ± 0.138 ^{a_A}	0.099 ± 0.002 ^{b_B}	0.00298
	AB	0.127 ± 0.002 ^{b_C}	0.301 ± 0.003 ^{a_A}	0.085 ± 0.004 ^{c_C}	0.00000519
	SEM	0.00000255	0.00224	0.00000432	

Location: DC, Domesticated; NB, New Benin Market; U, Uwa Market; AB, Arbico Market. SEM: Standard Error of Mean. a, b, c, superscripts within rows indicated organs are statistically significant. A, B, C, D subscripts within columns indicated locations are statistically significant. Mean with different scripts are significantly different at $P < 0.05$. Mean with the same scripts are not significantly different at $P > 0.05$.

Table 3. Mean concentrations of arsenic (mg/kg) in fresh kidney, liver and muscle of grasscutters from different locations on dry matter basis.

Variable	Location	Organs			SEM
		Kidney	Liver	Muscle	
As	DC	ND	ND	ND	ND
	NB	ND	ND	ND	ND
	U	ND	ND	ND	ND
	AB	ND	ND	ND	ND
	SEM	ND	ND	ND	ND

ND: Not Detected. Location: DC, Domesticated; NB, New Benin Market; U, Uwa Market; AB, Arbico Market. SEM: Standard Error of Mean.

muscle samples of grasscutters from New Benin market location was higher than the permissible limit (1.0 mg/kg) of chromium in meat samples set by European Commission (2006). The value observed is similar to levels reported by Iwegbue et al. (2008) showing that Cr concentration in turkey meat and chicken meat ranged between 0.01 and 3.43 mg/kg, and 0.01 and 4.83 mg/kg respectively. In an earlier study carried out by Khalil (2010), it was found out that Cr in fish and beef samples ranged between 73.4 and 54.05 mg/kg respectively. In contrast, the value observed is higher than that reported by Piskorová et al. (2003) from the muscle (0.02 to 0.49 mg/kg) (wet weight) of wild boars harvested in a polluted region of the Slovak republic in Italy. However, the smoke-dried muscle concentration was lower than the permissible limit (1.0 mg/kg). The value obtained is in agreement with Bratakos et al. (2002), which reported that the chromium content of pork ranged from 0.05 to 0.14 mg/kg (wet weight). The chromium levels recorded in this study shows that fresh muscle sample was also higher than Demirezen and Uruc (2006) permissible limit for Cr in food which reported generally 0.5 mg/kg. The chromium level in smoke-dried muscle was also lower than Demirezen and Uruc (2006). The high value reported in this study, may be most likely due to the accumulation of chromium from the environment. However, chromium is an essential mineral which is

useful at low concentrations, helping the body to use sugar, protein and fat. At high concentrations however, it is carcinogenic for organisms when present in the form of chromate (Suseno, 2015; Institute of Medicine, 2002). Excessive amounts of Cr may cause adverse effects on both animals and human (ATSDR, 2011). Hence, chromium is fairly evenly distributed throughout various foods, with the highest concentration found in meat. On the whole the mean Cr levels in the tissues examined is lower than the limit allowed and so fresh and smoke-dried grasscutter meat sold in New Benin market can be said to be relatively safe for consumption.

Arsenic and Nickel

It can be observed in Tables 3 and 4 that Arsenic concentration (mg/kg or ppm) was not detected in the kidney, liver and muscle of fresh and smoke-dried grass cutters sold at Arbico, Uwa, New Benin and Domesticated location. This may imply that the animal is not being affected or that the areas have very low arsenic metal pollution. Data obtained from Tables 5 and 6 show that Nickel concentrations (mg/kg or ppm) were highest ($P < 0.05$) in the muscle of fresh and smoke-dried grasscutters sold at New Benin market than in other locations. In comparing, the Nickel concentrations in both

Table 4. Mean concentrations of arsenic (mg/kg) in dried kidney, liver and muscle of grasscutters from different locations on dry matter basis.

Variable	Location	Organs			SEM
		Kidney	Liver	Muscle	
As	DC	ND	ND	ND	ND
	NB	ND	ND	ND	ND
	U	ND	ND	ND	ND
	AB	ND	ND	ND	ND
	SEM	ND	ND	ND	ND

ND: Not Detected. Location: DC, Domesticated; NB, New Benin Market; U, Uwa Market; AB, Arbico Market. SEM: Standard Error of Mean.

Table 5. Mean concentrations of nickel (mg/kg) in fresh kidney, liver and muscle of grasscutters from different locations on dry matter basis.

Variable	Location	Organs			SEM
		Kidney	Liver	Muscle	
Ni	DC	0.078 ± 0.002 ^{b_D}	0.087 ± 0.003 ^{a_B}	0.041 ± 0.001 ^{b_D}	0.00000183
	NB	0.109 ± 0.004 ^{c_C}	0.366 ± 0.021 ^{a_B}	0.725 ± 0.007 ^{a_A}	0.000075
	U	0.178 ± 0.012 ^{a_B}	0.181 ± 0.006 ^{c_C}	0.087 ± 0.006 ^{b_C}	0.000034
	AB	0.261 ± 0.004 ^{b_A}	0.465 ± 0.006 ^{a_A}	0.144 ± 0.009 ^{b_B}	0.0000189
	SEM	0.0000212	0.0000585	0.0000179	

Location: DC, Domesticated; NB, New Benin Market; U, Uwa Market; AB, Arbico Market. SEM: Standard Error of Mean. a, b, c, superscripts within rows indicated organs are statistically significant. A, B, C, D subscripts within columns indicated locations are statistically significant. Mean with different scripts are significantly different at P < 0.05. Mean with the same scripts are not significantly different at P > 0.05.

Table 6. Mean concentrations of nickel (mg/kg) in dried kidney, liver and muscle of grasscutters from different locations on dry matter basis.

Variable	Location	Organs			SEM
		Kidney	Liver	Muscle	
Ni	DC	0.071 ± 0.004 ^{b_C}	0.097 ± 0.003 ^{a_C}	0.045 ± 0.004 ^{c_C}	0.0000056
	NB	0.103 ± 0.003 ^{c_B}	0.159 ± 0.002 ^{b_B}	0.188 ± 0.001 ^{a_A}	0.00000199
	U	0.114 ± 0.005 ^{b_A}	0.163 ± 0.005 ^{a_B}	0.063 ± 0.004 ^{b_B}	0.00000989
	AB	0.076 ± 0.002 ^{b_C}	0.180 ± 0.001 ^{a_A}	0.050 ± 0.001 ^{c_C}	0.00000047
	SEM	0.00000566	0.0000039	0.00000377	

Location: DC, Domesticated; NB, New Benin Market; U, Uwa Market; AB, Arbico Market. SEM: Standard Error of Mean. a, b, c, superscripts within rows indicated organs are statistically significant. A, B, C, D subscripts within columns indicated locations are statistically significant. Mean with different scripts are significantly different at P < 0.05. Mean with the same scripts are not significantly different at P > 0.05.

fresh and smoke-dried samples the concentration of Ni in the kidney, liver and muscle were significantly and generally lower in the smoke-dried samples than the fresh ones. The highest concentration level of nickel was observed in the fresh and smoke-dried muscle of grasscutters (0.725 and 0.188 mg/kg) from New Benin market respectively, while the lowest concentration of (0.041 and 0.045 mg/kg) from the Domesticated location respectively was found in the fresh and smoke-dried muscle of grasscutters.

The concentrations of Nickel revealed that the value of the fresh and smoke-dried muscle from New Benin

market was higher than the tolerance limit (0.05 mg/kg) as earlier reported (Galadima and Garba, 2011) set by World Health Organisation. It was observed that the concentration levels of fresh and smoke-dried liver samples were higher than fresh and smoke-dried kidney samples and fresh and smoke-dried muscle samples were lowest with the exception of fresh and smoke-dried muscle sample from New Benin market location. This finding is similar to the observation of Flanjak and Lee (1979) who reported 0.33 ± 0.54 mg/kg Ni and 0.46 ± 0.87 mg/kg Ni in the liver and kidney of cattle respectively. Although, Korenekova et al. (2002) reported

Table 7. interactive comparison of chromium, arsenic and nickel concentrations (mg/kg) between fresh and dried tissues of grasscutters on dry matter basis.

Organ	Fresh			Dried			SEM
	Cr	As	Ni	Cr	As	Ni	
Kidney	0.2658 ^c	ND	0.1563 ^c	0.1493 ^c	ND	0.0911 ^c	0.0167
Liver	0.4393 ^c	ND	0.1498 ^c	0.2246 ^c	ND	0.1498 ^c	0.0371
Muscle	0.4130 ^b	ND	0.2491 ^b	0.1408 ^b	ND	0.0862 ^b	0.0666

ND: Not Detected. SEM: Standard Error of Mean. Means with different superscripts (a, b, c) within rows are significantly different at $P < 0.05$.

mean concentrations of 0.176 to 0.231 mg/kg in the liver of cattle reared in the vicinity of the metallurgic industry in Slovakia. Nickel is possibly an essential metal for experimental animals. Moreover, Nickel accumulates in the lungs and may cause bronchial hemorrhage or collapse and this agrees with the findings of the findings of Nielsen (1977). From the present results, it can be concluded that grasscutter under reference are unsafe for consumption with respect to Ni toxicity. Interestingly, it is also seen in Tables 3 and 4, that there were no concentration levels of Arsenic in all the tissues and locations.

Interactions

The concentration levels of Cr and Ni found in the fresh and smoke-dried liver samples from the different locations were generally higher than the concentration levels of these heavy metals in the fresh and smoke-dried kidney and muscle samples (Table 7). This is an indication that these heavy metals accumulate more in the liver than in the kidney and muscle. However, there are a few exceptions, where the concentrations of these heavy metals are higher in the kidney and in the muscle. It is a favorable condition that lead was not detected in all the samples of the domesticated grasscutter location. This may probably be attributed to method of capture or capture procedure, good monitoring and uncontaminated feed or drinking water as well as low environmental pollution near the breeding location or area. There was no Arsenic (As) concentration levels in all the samples investigated. Generally, all heavy metal concentrations in the smoke-dried kidney, liver and muscle reduced. This could be attributed to the report by Ahmed et al. (2011) who suggested heavy metal as being water borne which could explain a dripping deposition resulting from the washing of the meat prior to smoking. The drop in concentration of heavy metal in the smoked samples was also corroborated by Ajani et al. (2013) and Eboh et al. (2006) who attributed it to the fact that heat could have a significant effect on heavy metals and a possibility of the heavy metal being converted to other compounds.

CONCLUSION

The study showed that Cr and Ni concentration levels in the fresh and smoke-dried kidney, liver and muscle samples of the domesticated grasscutter location contained lower levels of the heavy metals than wild grasscutter market locations. Therefore, it is concluded that grasscutter domestication is another dimension in the livestock industry that has the potential to provide and ensure good quality and safety of grasscutter meat for the individual consumer health, bush meat processors, bush meat hunters and their families.

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